Trends in Microbiology

CellPress

Review

Cyanobacteria and biogeochemical cycles through Earth history

Patricia Sánchez-Baracaldo,[1,*] Giorgio Bianchini,[1] Jamie D. Wilson,[2] and Andrew H. Knoll[3]

Cyanobacteria are the only prokaryotes to have evolved oxygenic photosynthesis, transforming the biology and chemistry of our planet. Genomic and evolutionary studies have revolutionized our understanding of early oxygenic phototrophs, complementing and dramatically extending inferences from the geologic record. Molecular clock estimates point to a Paleoarchean origin (3.6–3.2 billion years ago, bya) of the core proteins of Photosystem II (PSII) involved in oxygenic photosynthesis and a Mesoarchean origin (3.2–2.8 bya) for the last common ancestor of modern cyanobacteria. Nonetheless, most extant cyanobacteria diversified after the Great Oxidation Event (GOE), an environmental watershed ca. 2.45 bya made possible by oxygenic photosynthesis. Throughout their evolutionary history, cyanobacteria have played a key role in the global carbon cycle.

Highlights

The core proteins of PSII involved in oxygenic photosynthesis originated during the early Archean, well before the GOE occurring around 2.3 billion years ago.

Most extant cyanobacterial taxa, including the lineage leading to chloroplasts, diversified after the GOE.

The evolution of modern planktonic cyanobacteria and phytoplanktonic algae reached global prominence at the end of the Precambrian, and they continue to significantly contribute to the carbon cycle.

Prior to the origin of complex life, cyanobacteria were the main primary producers during most of the Proterozoic Eon.

A new era in understanding the early evolution of cyanobacteria

Cyanobacteria are among the most important organisms ever to have evolved on our planet [1,2]. Their ancestors evolved the capacity for oxygenic photosynthesis, making possible the oxygenation of Earth's atmosphere and oceans. Moreover, cyanobacterial endosymbionts gave rise to photosynthesis in eukaryotes [3], implicating these bacteria in the overwhelming majority of all primary production on the modern Earth. Our understanding of oxygen's planetary history comes largely from studies of the geologic record [4–6]. Multiple lines of geochemical evidence indicate that oxygen (O_2) first became a permanent constituent of the atmosphere and the surface (but not deep) ocean about 2.45 Ga, during an event known as the Great Oxidation Event (GOE) [6,7]. As oxygenic photosynthesis provides the only source of oxygen sufficient to accomplish this environmental transformation, the GOE sets a minimum date for the origin of both this metabolism and the divergence of cyanobacteria from their closest nonphotosynthetic relatives. An increasing array of geochemical proxies suggests that a biogenic source of molecular oxygen existed much earlier, creating transient, localised oxygen oases by 2.7 Ga if not earlier [8] (Figure 1). Multiple hypotheses exist to reconcile such geochemical data (reviewed in [9]), but a fuller understanding of the interplay between life and environment on the early Earth requires information that Earth science alone cannot provide. When, for example, did oxygenic photosynthesis first evolve? When did the Cyanobacteria crown group appear? And how did biological innovations in this group contribute to major transitions in biogeochemical cycles? Some of these questions are still hugely debated yet they are key to interpreting the geological record.

Life has recorded its own history within genomes, and this genomic record can be used to elucidate the appearance of bacterial metabolisms [10]. Through phylogenetic analyses and genomic data, recent studies have shed new light on the early evolutionary history of cyanobacteria and the core proteins involved in water oxidation [11]. Genomics and evolutionary studies have also identified the time of origin for planktonic groups that likely increased marine primary productivity in early oceans [12–15]. Such biological events would have played a part in the emergence of a strong biological pump, influencing most of Earth's key biogeochemical cycles. During the last

[1]School of Geographical Sciences, University of Bristol, Bristol BS8 1SS, UK
[2]School of Earth Sciences, University of Bristol, Bristol, BS8 1SS, UK
[3]Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138, USA

*Correspondence: p.sanchez-baracaldo@bristol.ac.uk (P. Sánchez-Baracaldo).

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decade, increased availability of cyanobacterial genomes has allowed us to explore biological evidence that illuminates the origin of oxygenic photosynthesis and the radiation of different lineages that have proliferated throughout the sunlit biosphere [11]. Here we review key studies that have advanced our current understanding of major cyanobacterial groups and how they might have impacted biogeochemical cycles. We also discuss remaining inconsistencies between the genomic and geological records, addressing some outstanding and controversial questions.

The early origin of oxygenic photosynthesis

Eight known extant bacterial phyla contain groups with photosynthetic reaction centres (RCs). These photosynthetic lineages include Cyanobacteria, Proteobacteria, Chloroflexi, Acidobacteria, Chlorobi, Firmicutes, Gemmatimonadetes, and *Candidatus* Eremiobacterota [16,17]. Ancestral prokaryotes evolved mechanisms to covert light energy into chemical energy [2] using electron donors other than water (H_2O) [17]. The availability of electron donors likely limited primary production by early anoxygenic photoautotrophs [17,18], providing strong selection pressure for bacteria able to extract electrons from readily abundant water. Stem group oxygenic phototrophs [11] in early environments were probably limited by both the bioavailability of phosphorus [9] and competition from anoxygenic photoautotrophs in environments where electron donors other than H_2O were available [19–21]. Over time, oxygenic photosynthesis became the main source of energy for life on Earth, largely removing alternative electron donors from waters within the photic zone [6,11].

Oxygenic phototrophs have two photosystems – **Photosystem I (PSI**) and **Photosystem II (PSII**) (see Glossary) – in contrast to the single photosystem (either PSI or PSII-like) found in anoxygenic photoautotrophs [1]. Structural and sequence analyses of reaction centre proteins indicate that these molecules share a common ancestor across all phototrophs [22]. This phylogenetic pattern implies that modern photosynthetic species evolved from a single ancestor – yet, by looking at the bacterial tree of life, extant phototrophs do not share a common ancestor that was, itself, phototrophic [16]. These discrepancies show that more research is required to tease apart what evolutionary mechanisms led to the documented photosynthetic diversity among bacteria (e.g., lateral gene transfer, gene duplication and differential loss).

Comparative structural biology and phylogenetic analyses have brought insights into the evolutionary trajectory, leading to the recognition of a functional photosynthetic RC, capable of evolving oxygen, and pointing to water oxidation and the origin of oxygenic photosynthesis early in the history of life [2,11,23]. These findings also imply that anoxygenic and oxygenic phototrophs coexisted in time but were separated by environment for most of the Archean Eon, prior to the rise of cyanobacteria to ecological dominance.

Molecular clock estimates for the gene family that includes PSI and PSII [2,11,23] are consistent with evidence showing traces of oxygen throughout the Archean Eon (4–2.5 Ga). It is therefore probable that oxygenic photosynthesis was already established by 3.0 Ga, if not earlier [6,24] (Figure 1). Indeed, early forms of water oxidation, carried out by ancestral homodimeric photosystems, could have originated a billion years before the GOE [2]. The highly sophisticated PSII found in extant cyanobacteria can be traced back to the Mesoarchean (3.2–2.8 Ga) [2].

How can we reconcile molecular and geochemical evidence for the early evolution of oxygenic photosynthesis with the much later GOE? Important clues come from experiments [21] and ecological studies [25] which show that, where alternative electron donors are available, anoxygenic photosynthetic bacteria commonly outcompete cyanobacteria. With the strong phosphorus (P)

Glossary

Benthic: a term used here to refer to habitats in which microbes attach to surfaces such as sediments and rocks beneath water bodies.
Biological carbon pump: the creation of plankton-derived organic matter in the surface ocean and its subsequent fate in the ocean interior as particles (organic detritus, faecal pellets) sink out of the photic zone. The net effect is to sequester carbon within the ocean, redistribute oxygen demand throughout the ocean interior, and deliver organic matter to the seafloor for burial.
Filamentous: refers to long strands of bacterial cells growing end to end and interlocking with each other to form a network.
Heterocysts: specialised cells uniquely found in cyanobacteria. Vegetative cells differentiate into cells that contain additional wall layers and modified thylakoids that lack a functional PSII. These cells, which are sites of nitrogen-fixation, are characteristic of the Nostocales.
Macrocyanobacteria: a monophyletic clade containing lineages with cell diameters ranging from 3 to 50 μm.
Microbial carbon pump: the creation and respiration of plankton-derived dissolved organic matter (proteins, carbohydrates, lipids). This pump sequesters carbon in the form of recalcitrant DOM that accumulates in the ocean interior.
Microcyanobacteria: a monophyletic clade containing lineages with small cell diameters (<3 μm). This clade is sister to the Macrocyanobacteria.
Multicellularity: refers to filamentous species capable of processes such as cell–cell adhesion, intercellular communication, and cell differentiation, resulting in vegetative cells, akinetes, and heterocysts.
Photosystem I (PSI): a protein complex that is the second one in the light-dependent reactions of oxygenic photosynthesis; it transfers electrons across the thylakoid membrane from plastocyanin to ferredoxin. PSI produces the high-energy carrier NADPH that is used for the subsequent photosynthetic dark reaction or Calvin cycle.
Photosystem II (PSII): a protein complex that is the first one in the light-dependent reactions of oxygenic photosynthesis; it is located in the thylakoid membrane. Enzymes, in the complex, capture photons of light to

limitation modelled for Archean oceans [9], many environments would have become depleted in P before they ran out of ferrous iron (Fe^{2+}), hydrogen sulfide (H_2S), or hydrogen (H_2), limiting oxygenic photosynthesis and, hence, oxygen production [19]. Environments poor in alternative electron donors would have existed in the Archean, however, providing niche space for oxygenic photoautotrophs. Moreover, some extant cyanobacteria can switch to anoxygenic photosynthesis when H_2S is available; such metabolic flexibility could have been important on the early Earth [26]. Tectonically driven increases in P and decreases in Fe^{2+} fluxes into the oceans provide physical drivers for the rise of cyanobacteria to ecological prominence and the environmental transformation of the GOE.

excite electrons that are transported through a range of coenzymes and cofactors to reduce plastoquinone to plastoquinol. Excited electrons are replaced by the oxidation of water, resulting in the production of molecular oxygen and hydrogen ions.
Phytoplankton: algae and cyanobacteria which grow free-floating in the water column, in both marine and freshwater habitats.

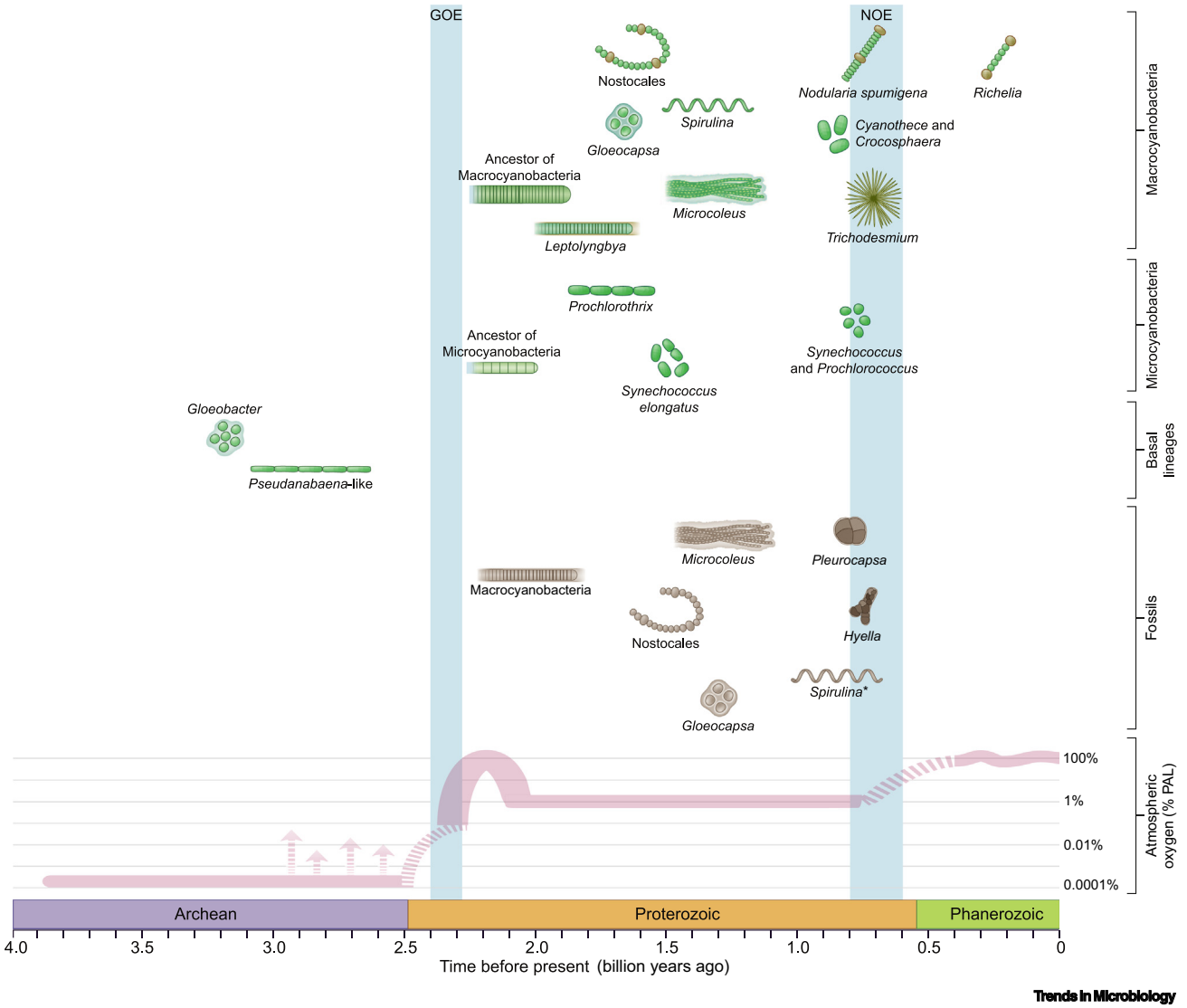

Figure 1. Timeline of oxygen concentration in the atmosphere, the antiquity of cyanobacterial lineages based on molecular clock estimates and fossil occurrences. Age estimates for crown group Cyanobacteria and major clades and taxa from [27]; oxygen curve modified from [8]. Modern cyanobacteria are shown in green, and fossils in brown; these cartoons are not drawn according to scale. Taxa with smaller cell diameter (basal lineages and Microcyanobacteria) are shown at the bottom, and those with larger cell diameter (Macrocyanobacteria) at the top. Major cyanobacterial clades radiated into many diverse forms after the Great Oxidation Event (GOE). Marine planktonic cyanobacteria evolved towards the end of the Proterozoic Eon and again in the Cretaceous Period. The asterisk marking *Spirulina* indicates that these helical fossils could alternatively belong to *Arthrospira*. This figure has been modified from Figure 3 in Sánchez-Baracaldo and Cardona [11] with oxygen data from Lyons *et al* [6].

The origin of the Cyanobacteria crown group

The increasing number of cyanobacterial genomes (~950 genomes) and metagenomic data have significantly improved our understanding of the wider diversity and evolutionary relationships of extant cyanobacteria [13,27,28], although there are still a large number of under-represented taxa (e.g., from polar regions, drylands, freshwater lakes). Phylogenomic analyses reveal that the closest relatives of cyanobacteria are the nonphotosynthetic taxa: Vampirovibrionia (formerly Melainabacteria) [29] and Sericytochromatia [30] (Figure 2). Several hypothesis have been put forward explaning why Cyanobacteria are sister to non-photosynthetic living relatives. Some authors have hypothesised that cyanobacteria acquired their photosynthetic machinery via lateral gene transfer after their split from the ancestor shared with Vampirovibrionia [30]. In contrast, others have proposed differential gene loss, postulating that oxygenic photosynthesis was present in a bacterial common ancestor but was lost in some lineages as they diversified and specialised into new niches [2,31]. Such contrasting explanations for the broader phylogenetic pattern of photosynthetic lineages amongst nonphotosynthetic taxa continue to be debated [11,31].

About a decade ago, most available genomic data for the cyanobacteria were biased toward marine unicellular taxa (e.g., *Synechococcus* and *Prochlorococcus*) [32]; this has changed within the last 8 years as a number of studies have expanded genomic representation from previously undersampled freshwater and terrestrial habitats [28]. Comparative genomic analyses are now revealing how constraints imposed by the environment have shaped the evolution and genomic structure of cyanobacteria from land, freshwater, and marine habitats [28]. Some early cyanobacterial lineages have likely gone extinct, which means that some phylogenetic signal

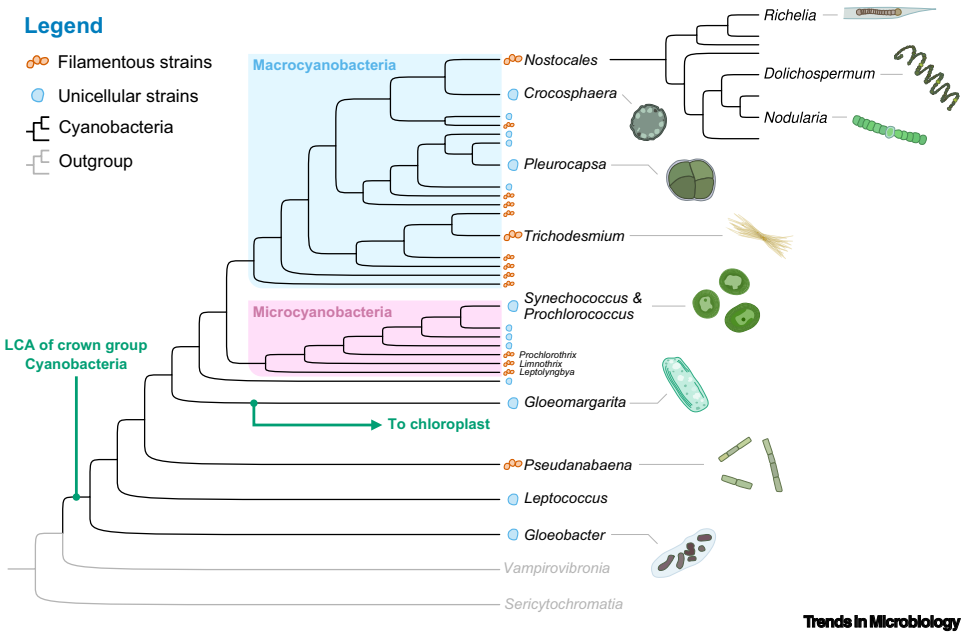

Figure 2. Cyanobacteria tree of life. A cladogram representing the main cyanobacterial groups and their closest nonphotosynthetic relatives (Vampirovibrionia and Sericytochromatia). Deep branching relationships of Cyanobacteria are based on phylogenetic analyses, including 139 proteins and two ribosomal RNAs – small subunit (SSU) and large subunit (LSU) [27]. For each cyanobacterial group, it is shown whether it contains filamentous or unicellular forms. The last common ancestor (LCA) of crown group Cyanobacteria, and the position of the ancestor of chloroplasts, are highlighted. Branch lengths are in arbitrary units.

has been obscured. The continued discovery of early branching lineages and a multilevel approach (e.g., see study on phycoerythrin genes combining comparative genomics, phylogenomic, and single-gene phylogeny approaches [13,14]) will likely help to piece together early events in the evolution of photosynthesis and photosynthetic organisms [11].

Using molecular clocks to estimate the age of the Cyanobacteria crown group is intrinsically difficult, in part because of limited fossil calibrations near the root (Box 1). One solution has been to rely exclusively on calibrations from photosynthetic eukaryotes [33], although this necessitates the assumption that rates of sequence evolution in chloroplasts approximate those of free-living cyanobacteria (see [34]). The good news is that cyanobacteria include some of the largest, most morphologically diverse, and most preservable of all prokaryotes, with growth forms that range from simple unicells to multicellular structures characterized by cell differentiation. The bad news is that at least some of the simpler morphologies have evolved independently in multiple parts of the tree [35,36]. Fortunately, at least some Proterozoic microfossil populations display morphologies that can be assigned to specific branches in cyanobacterial phylogeny, for example, endolithic pleurocapsalean taxa that display complex branching (i.e., cells arranged in rows that form pseudo-filaments within sheaths) and behaviour [37], and nostocalean populations that preserve akinetes and short trichomes closely comparable to germlings in close physical association [38]. And if we accept the existence of a macrocyanobacterial clade containing all taxa larger than 3 μm ([13], see following text), fossils constrain the origin of this clade to >2.015 Ga [39]. Reports of earlier fossil cyanobacteria remain contentious [40,41].

In recent age estimates for the divergence of Cyanobacteria from their closest relatives, Vampirovibrionia, the median ages range between ~2.5 Ga [33] and ~3.1–3.3 Ga [27] (Table 1). Discrepancies in age estimates for the Cyanobacteria crown group are likely due to a

Box 1. Dating the last common ancestor of cyanobacteria

Estimating the timing of bacterial origins is challenging due in part to the lack of fossil calibrations. Cyanobacteria have arguably one of the best fossil records amongst bacterial groups, with their distinct morphology, abundance in the fossil record, and resemblance to extant taxa. Taking advantage of this, many studies have implemented state-of-the-art techniques to study the timetable of cyanobacterial evolution, using 'relaxed molecular clock' approaches which combine phylogenetic analyses with dated fossil calibrations and Bayesian approaches [100]. Whilst cyanobacterial fossils can be identified in Proterozoic rocks, relatively few can be assigned to a specific phylogenetic position with confidence, and as we recede further back in time, the abundance of fossils and confidence in their interpretation decline markedly.

Bayesian approaches enable one to take into account uncertainty in molecular clock analyses: these combine the data from the phylogenetic analysis and the fossil calibrations, with a 'prior distribution' describing the chosen fossil calibration based on a specific criterion. For instance, based on the geochemical record, it assumed that cyanobacteria already existed by ~2.4 bya when the GOE occurred [36]. The result is a 'posterior distribution' that reflects the amount of uncertainty in the inference process [101]. The results of a Bayesian analysis may or may not depend strongly on the chosen prior distribution; for instance, when large amounts of data are consistent with the same hypothesis, the choice of prior can be largely inconsequential. In contrast, if the data are scarce and/or contradictory, different priors can produce vastly different results [101]. This is the case for time estimation of the last common ancestor of cyanobacteria and its divergence from its closest nonphotosynthetic relatives. Several factors could contribute to the lack of data, such as extinction of basal lineages, and lack of reliable bacterial fossils near the root, amongst others. The age estimation of the cyanobacterial common ancestor greatly varies with different priors (Figure I) highlighting the need for more research into cyanobacterial fossils, discovery of basal lineages, and increasing the resolution of the relationships between extant lineages.

The fossil record of eukaryotes can aid the calibration of cyanobacterial phylogeny since the chloroplasts of algae and land plants are descended from once free-living cyanobacteria [102]. It is sometimes possible to include eukaryotic fossils in molecular clock analyses of cyanobacteria, in what is called 'cross-calibration'. Indeed, some researchers purposely exclude all bacterial fossils from their analyses, including only cross-calibrations with eukaryotic fossils, which they deem to be more reliable than their bacterial counterparts [33].

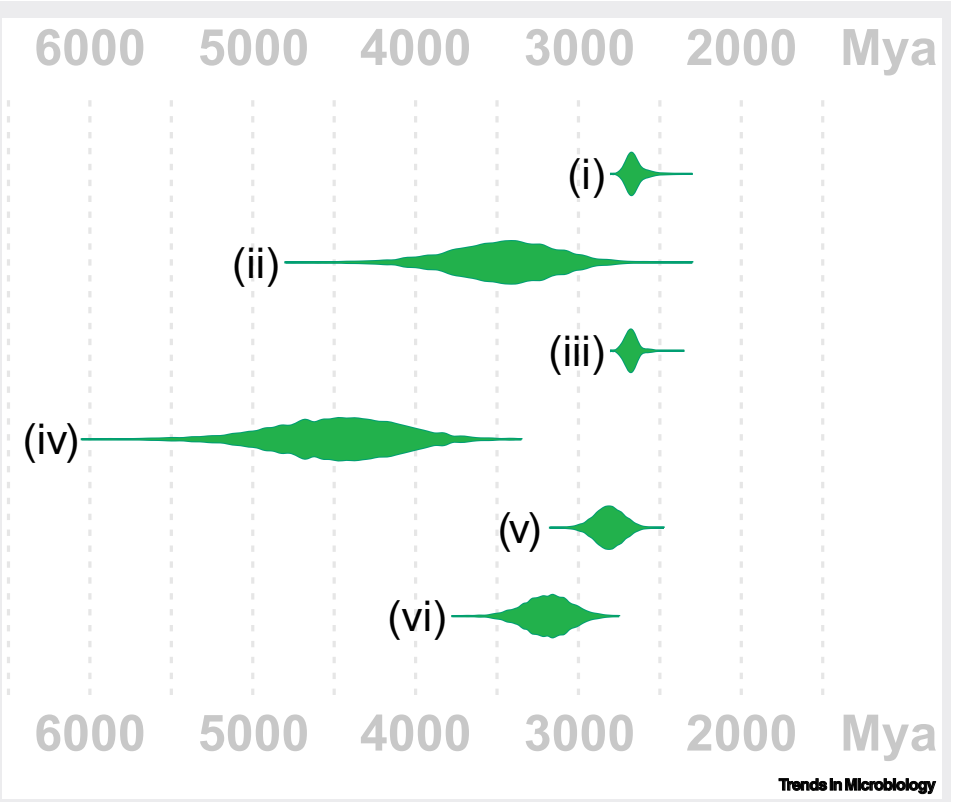

Figure I. Posterior age estimates for the last common ancestor of crown group Cyanobacteria. The figure shows posterior distributions for the estimate of the age of the last common ancestor of crown group Cyanobacteria. The posterior distributions were obtained using the same dataset but varying the outgroup and the prior. (i) Terrabacteria (Chloroflexi, Firmicutes, Tenericutes) outgroup, 3060 ± 404 million years ago (mya) root calibration, 2510 ± 190 mya Cyanobacteria calibration. (ii) Terrabacteria (Chloroflexi, Firmicutes, Tenericutes) outgroup, 3060 ± 404 mya root calibration, without prior Cyanobacteria calibration. (iii) Vampirovibrionia outgroup, 3060 ± 404 mya root calibration, 2510 ± 190 mya Cyanobacteria calibration. (iv) Vampirovibrionia outgroup, 3060 ± 404 mya root calibration, without prior Cyanobacteria calibration. (v) *Gloeobacter* outgroup, 2500 ± 100 mya root calibration. (vi) *Gloeobacter* outgroup, 2639 ± 179 mya root calibration. All calibrations had soft bounds.

lack of implementation of fossil calibrations, taxon sampling, and the choice of relaxed molecular clock models [15] (Box 1). Published age estimates that have been reported vary from before [13,27,42–44] to after [33] the GOE (Table 1).

Early diversification within the cyanobacteria

During the past decade, large-scale phylogenetic analyses have resolved the deep-branching relationships of cyanobacteria [13,28]. The earliest diverging forms were unicellular, with small cell diameter (e.g., *Gloeobacter, Synechococcus* from Octopus Spring) [13,32,45]. Additionally, metagenomic data have recently revealed an uncultured freshwater cyanobacterium (*Candidatus* Aurora vandensis) from Lake Vanda in Antarctica, which plots as sister to *Gloeobacter* spp. [46] (see Outstanding questions).

During the late Archean, cyanobacteria diversified into a wide range of habitats, from terrestrial to low- and high-salinity aqueous environments [13,32,47]. *Pseudanabaena*-like **filamentous** forms diverge near the root of the cyanobacterial tree [13,28,36], prompting the proposal that the emergence of

Table 1. Molecular clock age estimates for cyanobacteria and their closest relatives. Comparison of relaxed molecular clock studies estimating the age of origin for the Cyanobacteria crown group and the Stem group (divergence between cyanobacteria and their nonphotosynthetic relatives, Vampirovibrionia and Sericytochromatia). Median ages and 95% confidence interval in bya.

Cyanobacteria crown group	Time period	Refs
3.63 (3.87–3.39)	Paleoarchean	[43]
2.64 (2.72–2.48)	Neoarchean	[27]
2.67 (2.42–2.97)	Neoarchean	[44]
2.71 (2.89–2.53)	Neoarchean	[13]
2.02 (1.72–2.37)	Paleoproterozoic	[33]
Stem Cyanobacteria		
3.37 (4.05–2.80)	Paleoarchean	[27]
3.15 (2.88–3.48)	Mesoarchean	[44]
2.54 (2.09–3.06)	Neoarchean	[33]

multicellularity contributed to a significant increase in diversification rates around the GOE [36,48]. Filamentous forms were likely a significant biological innovation that would have facilitated the formation of microbial mats and biofilms [32]. The geologic record shows that, throughout the Proterozoic Eon, microbial mats were widespread on lake margins and the sunlit seafloor [49], quite possibly reflecting the early diversification of filamentous lineages [13,32].

Several other innovations evolved around the GOE, including an increase in cell diameter [32] in the ancestors of **Macrocyanobacteria**, a clade that includes taxa with cell diameters >3 μm up to 50 μm, encompassing the greatest taxonomic diversity of extant cyanobacteria [13]. The evolution of larger cell diameters is significant, as most prokaryotes have cell diameters of the order of 1 μm. Furthermore, its timing might help to explain why the earliest unambiguous cyanobacterial microfossils are found, as noted previously, after the GOE [36,50]. Derived lineages within the Macrocyanobacteria evolved cell differentiation and specialised labour within the Proterozoic Eon [51], again documented by fossils as old as 1.5 Ga [38]. In fact, most extant cyanobacterial groups originated after the GOE [32] from ancestors belonging to the Macro- and **Microcyanobacteria** (Figure 2).

The genomic record shows no evidence of planktonic cyanobacteria amongst early branching cyanobacterial strains, although, as discussed in the following text, it is possible that early planktonic groups left no descents or are yet to be discovered. Phylogenetic and molecular clock analyses, however, imply that, during the early and mid-Proterozoic, **benthic** microbial mats would have been major players in global primary production.

The biological legacy of cyanobacteria

In the modern world, cyanobacteria are diverse and nearly ubiquitous in the photic zone. They have had a further huge impact on biological diversity due to their ability to establish symbiotic relationships with a number of different hosts [52–54]. The most important of these was the incorporation of a basal cyanobacterium (closely related to *Gloeomargarita*) [15,55] (Figure 2) within a protistan host, giving rise to the clade of photosynthetic eukaryotes known as the Archaeplastida – glaucophytes, red algae, green algae, and land plants. Molecular clock analyses variously estimate this endosymbiotic event to have been established ~1.9–1.25 Ga [15,56].

The fossils record indicate that eukaryotes inhabited the world's oceans as early as 1.8–1.6 Bya [57], with unambiguous evidence for simple multicellular red and green algae by a billion years

ago [58–60], and, if stratigraphic interpretation is correct, perhaps somewhat earlier [61]. Later transfers of photosynthesis to other lineages of heterotrophic eukaryotes through eukaryote–eukaryote mergers (secondary and tertiary endosymbiosis) led to the (largely Phanerozoic) evolution of Earth's current photosynthetic diversity [62]. The unicellular alga *Paulinella chromatopora* documents a second, more recent, origin of plastids from an endosynbiotic cyanobacterial ancestor [63].

Beyond plastids, cyanobacterial symbioses are widely distributed in marine, freshwater, and terrestrial environments, involving taxonomically diverse hosts [54,64,65]. Cyanobacteria shape the biology and evolution of their hosts by providing a range of benefits such as a source of carbon, nitrogen-fixation, UV protection, and defensive toxins [64]. Symbionts are found across the cyanobacterial tree of life, including unicellular and filamentous, nitrogen-fixing and non-nitrogen-fixing species. Broadly speaking, in terrestrial ecosystems, symbioses are restricted to lichens and several groups of land plants (e.g., bryophytes, ferns, cycads), while in aquatic ecosystems, hosts involve sessile or slow-moving animals (e.g., sponges, ascidians) and a number of planktonic protists (e.g., diatoms, prymnesiophytes) [54,65]. Many of these cyanobacterial symbionts fix N_2, a feature that enables hosts to grow in areas deficient in bioavailable N [66]. For example, it has been estimated that the association between the diatom *Hemiaulus hauckii* and its nitrogen-fixing cyanobacterial symbiont *Richelia intracellulari* accounts for 89–100% of nitrogen fixation in the western tropical North Atlantic under bloom conditions [67].

The biogeochemical legacy of cyanobacteria

The principal biogeochemical consequence of cyanobacterial evolution is our oxygen-rich biosphere, but molecular clock estimates and corroborating geochemical evidence indicate that the evolution of oxygenic photosynthesis, per se, was not a magic wand that led immediately to biospheric transformation. Rather, early cyanobacteria coexisted in anoxic environments with anoxygenic photoautotrophs, rising to ecological prominence only as Earth's tectonic development shifted fitness landscapes [20]. While O_2 was established as a permanent component of the atmosphere and surface ocean during the GOE, its partial pressure remained modest – perhaps 1% or so of modern levels – for nearly two billion years after this event, beginning its rise to modern values only near the end of the Proterozoic Eon [6] (Figure 1). During the long Proterozoic Eon, cyanobacteria were the major primary producers in the oceans [68,69], ceding ecological prominence in continental shelf and platform environments only as renewed tectonic change transformed the phosphorus cycle, and with it, global oxygen levels [70–72]. Even today, however, cyanobacteria – principally the genera *Prochlorococcus* and *Synechococcus* – account for an estimated 25% of all photosynthesis in the oceans [73]. Through their roles in primary production and oxygen generation, cyanobacteria fundamentally transformed the cycling of carbon, nitrogen, sulfur, iron, and redox-sensitive trace metals, influencing the temporal and spatial evolution of diverse microorganisms (e.g., [74]).

Nitrogen cycling

Some cyanobacterial lineages are able to fix atmospheric nitrogen (N_2) into bioavailable ammonia (NH_3) or ammonium ion (NH_4^+). Nitrogen exerts a strong control on global primary productivity as a limiting nutrient in both marine and terrestrial environments, and as such has had a significant influence on the evolutionary development of the biosphere. Whilst nitrogen-fixation is found across a wide range of archaeal and bacterial lineages [75], in the surface oceans cyanobacteria are presumed to be the major N_2-fixing microorganisms [66]. Trait evolution analyses show that the acquisition of nitrogenase, the key enzyme in biological nitrogen fixation, occurred near the

root of the cyanobacterial tree [76]; genome sequencing has also revealed that nitrogenase is widely distributed across both unicellular and filamentous taxa. Nitrogenase, which catalyses rupture of the triple bond in N_2, is inhibited by molecular oxygen, but some taxa have evolved specialised cells called **heterocysts** that permit oxygen-sensitive N_2 fixation in oxic environments. Heterocysts evolved only once, in the Nostocales [47,77]. That said, diverse strategies have evolved to ensure the spatial separation of water oxidation and nitrogen fixation in cyanobacteria [66]. Moreover, genome sequencing has revealed the presence of nitrogenases in taxa previously thought to be incapable of nitrogen fixation, including some small-diameter unicellular taxa (i.e., *Synechococcus*) [78].

Significant cyanobacterial contributions to the Earth's nitrogen cycle would have occurred once free-living marine planktonic nitrogen fixers (e.g., *Trichodesmium*, *Crocosphaera* clade, *Nodularia spumigena*) evolved, toward the end of the Proterozoic Eon [12,13], approximately when geochemical data suggest an increase in bioavailable P (e.g., [72]). Further significant contributions to the marine N cycle would have occurred with the evolution of nitrogen-fixing cyanobacterial symbionts within planktonic algae, as noted previously. Recent genomic studies have also shown that mixotrophy, the ability to be able to use organic matter as a source of C and N, is widespread amongst cyanobacteria [79].

Primary productivity in a microbial mat world

Cyanobacteria derived from early branches live in nonmarine habitats or as benthos within the shallow, sunlit oceans. As noted previously, it may be that extinct cyanobacteria inhabited the planktonic realm early on, and both the distribution and carbon isotopic composition of organic matter among sedimentary facies in Archean and earlier Proterozoic sedimentary basins is consistent with the presence of planktonic photoautotrophs in early oceans [80,81]. That said, the quantitative contribution of cyanobacteria to global biogeochemical cycling during most of the Archean and Proterozoic eons would have been limited by nutrient availability. It is well known that the availability of N [4,82], P [83], and trace metals [4] exerts a strong control on primary production, and thus carbon burial and O_2 release into the atmosphere; this would have been certainly the case in Precambrian oceans, in which the inefficient remineralization of organic matter [84] and P sequestration into iron phosphates [85] would have strongly constrained primary production. Moreover, spatial modelling indicates that both productivity and pO_2 would have been particularly low in the ocean gyres where phytoplanktonic cyanobacteria thrive today [86]. Consistent with this, phylogenies suggest that early cyanobacteria and ancestral photosynthetic eukaryotes (e.g., basal red and green algae) lived mostly in terrestrial and marginal marine environments [15].

Phylogeny and the geologic record agree that, prior to the Neoproterozoic expansion of phytoplankton, the most widespread and productive cyanobacteria were (mostly) filamentous mat-forming forms and other - microbial benthos [13,32,36]. It is important to emphasise that microbial mats are highly productive ecosystems in which feedbacks between cyanobacteria and sulfate-reducing bacteria take place within a few densely packed vertical millimetres [87]. Although geobiological studies point to the widespread importance of microbial mat ecosystems on the Precambrian Earth, their quantitative contribution to global primary production may have been limited. While estimates of benthic oxygenic primary productivity exceed those for the pelagic realm by 1000–10000-fold on a per-volume basis [88], sunlit benthic habitats (including the terrestrial, biological soil crusts) occupy a small area in contrast to the open ocean. Therefore, the global biogeochemical impact of oxygenic photosynthesis may have increased markedly when cyanobacteria started colonizing the open ocean [12,13].

Primary productivity in a planktonic world

As noted previously, most extant marine planktonic cyanobacteria can be traced back to the Neoproterozoic Era [12–14,36]. In the context of all cyanobacterial diversity, only a handful of taxa inhabit the open ocean (i.e., *Crocosphaera* and relatives, cyanobacterium UCYN-A, *Trichodesmium*, *Prochlorococcus*, and *Synechococcus*). Biomarker [71] and phylogenetic [89] data point to a relatively late rise of photosynthetic eukaryotes to ecological prominence in the marine phytoplankton. The Neoproterozoic diversification of both planktonic cyanobacteria and eukaryotic **phytoplankton** marks a transition in organic carbon cycling dominated initially by dissolved organic carbon to a more modern-like cycle dominated by relatively rapid sinking and recycling of biogenic particulates within the ocean interior (Box 2, Figure 3). Interestingly, planktonic picocyanbacteria began their radiation at about the same time that eukaryotic phytoplankton first rose to ecological prominence [71], which, in turn, correlates with an increase in P availability in sunlit shelf waters and at least a moderate increase in pO_2 [6,70,72]. Thus, the rise of planktonic cyanobacteria capable of nitrogen fixation may reflect increased P availability [90] as well as the capacity of picoplanktonic *Synnechococcus* and some *Prochlorococcus* strains to utilize nitrate [91–93].

Prochlorococcus strains unable to metabolize nitrate are particularly adept at scavenging ammonium from seawater (e.g., [92]), and despite their limited diversity and small cell size, modern planktonic cyanobacteria are significant contributors to organic carbon cycling. They contribute 25% of net marine primary productivity due to an almost ubiquitous distribution of biomass across the global ocean [73,94]. Open-ocean phytoplankton mediating nitrogen

Box 2. Organic matter cycling: the biological and microbial carbon pumps

The biogeochemistry of carbon and oxygen is strongly influenced by the creation and fate of organic matter formed by plankton. Phytoplankton in the surface ocean fix carbon and other major elements into organic matter (primary productivity: 50–60 Gt C $year^{-1}$). Around <25% escapes respiration in the surface ocean [103]. The fate of this organic matter is separated into two loosely defined modes: the 'biological carbon pump' and the 'microbial carbon pump' (Figure I). These two modes reflect significant differences in the magnitude, timescale, and locus of respiration of organic matter in the ocean interior.

The biological carbon pump

Particles of organic matter (POM), formed of aggregated detritus and faecal pellets, sink from the ocean surface to be mostly respired at depth, leaving a small fraction reaching the seafloor to be buried [73,94]. The ocean circulation ultimately transports respired carbon and nutrients back to the surface but at much longer timescales (100–1000 years) than the redistribution by sinking particles (days). In the modern ocean, the biological pump sequesters ~1700 Pg C that would otherwise reside in the atmosphere, increasing CO_2 concentrations by 150–200 p.p.m. [104].

The microbial carbon pump

Plankton ecosystems also produce dissolved organic matter (DOM) from cell lysis and sloppy grazing that enters the ocean interior via circulation. The majority of DOM produced is labile and is rapidly respired in the upper water column (100–1000 m) but a fraction persists for up to 4000–6000 years forming a pool of ~700 Pg C [105]. The apparent recalcitrance is thought to be a function of refractory molecular structure [106], environmental factors such as temperature or concentration of oxidants [106], relative dilution of individual compounds [107], or some combination of factors.

Despite the low sinking speeds associated with typical cyanobacteria cell sizes of <2 μm, cyanobacteria contribute to contribute to the biological carbon pump [108]. Small cells aggregate into larger particles aided by exudates produced by picoplankton under nutrient stress [108]. As well as increased sinking velocity, these aggregates are also grazed by mesozooplankton incorporating the organic matter into dense fast-sinking faecal pellets [109]. Ecosystems dominated by picoplankton are potentially significant sources of refractory DOM [110] for the microbial carbon pump.

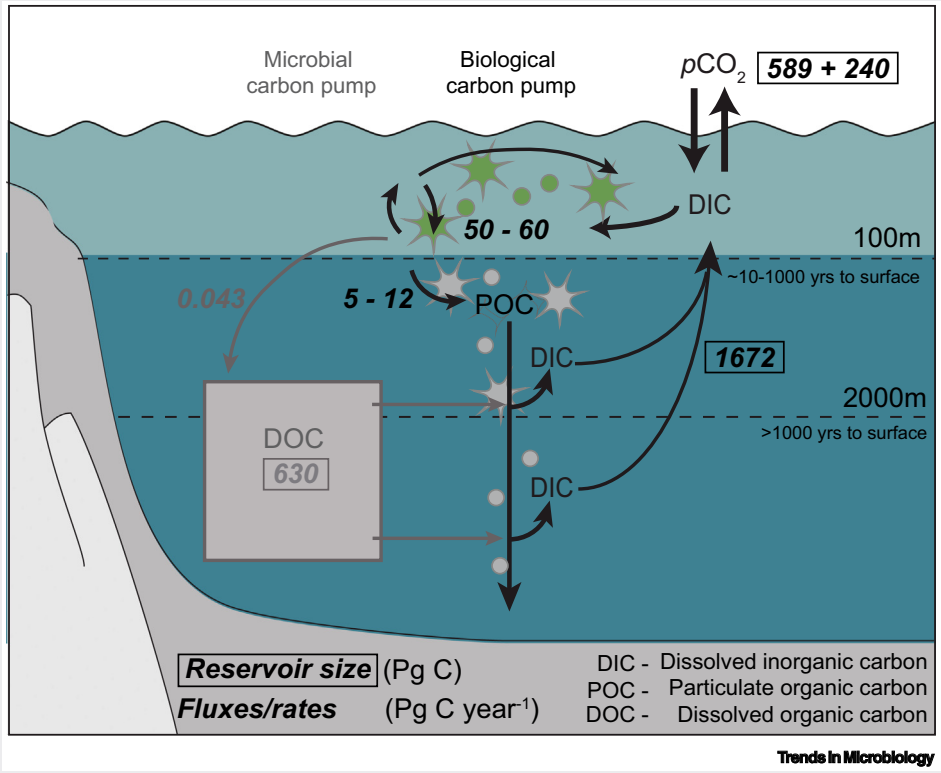

Figure I. Schematic depicting the biological and microbial carbon pumps in the modern ocean. The biological and microbial carbon pumps are depicted in black and grey, respectively. Values for the biological pump are taken from Henson *et al*. [103], Williams and Follows [111]. Values for the microbial carbon pump are given for the geologically relevant pool of refractory dissolved organic carbon from Hansell [105]. The reservoir size for the atmosphere shows preindustrial + anthropogenic contributions [112].

and carbon cycling likely played an important role in biospheric transformation via increased organic matter burial in marine sediments and atmospheric pCO_2 drawdown due to increased biological export to the ocean floor [95] (Figure 3). Increased oxygen availability as a result of increased carbon burial would, in turn, have fueled a positive feedback between nutrient generation and pO_2, helping to establish the distinct oxygen-rich Phanerozoic biosphere [72]. Increased nutrient availability led to plankton biomass levels sufficient to support more trophic complexity [96], increasing the proportion of export reaching the seafloor via larger cell sizes and aggregation, though ultimately limited by environmental factors such as oxygen and temperature [97].

Prior to the establishment of a near-modern biological pump, the cycling of plankton-derived organic carbon may have operated very differently [98]. The ineffective sinking of small cells coupled with slower degradation of organic compounds in an anoxic ocean facilitated the accumulation of dissolved organic carbon to significantly greater levels than in the modern ocean [99] (Box 2, Figure 3) (see Outstanding questions). Large perturbations in the Neoproterozoic carbon cycle may then reflect the diminution of the global DOC reservoir associated with large-scale changes in the environment and a shift towards organic matter cycling dominated by the biological pump [95].

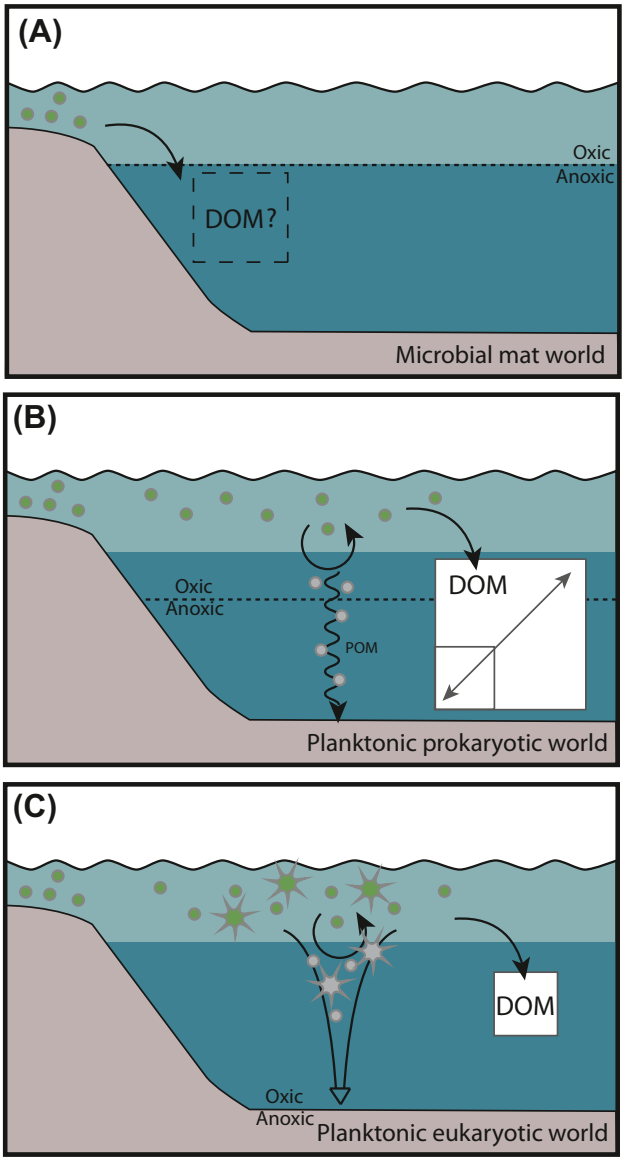

Figure 3. The relationship between cyanobacteria and global marine biogeochemical cycling through Earth's early history. (A) Primary production is dominated by microbial mats in shelf oceans. (B) Cyanobacteria colonise the open ocean, initiating a protobiological pump. Anoxic conditions lead to the accumulation of dissolved organic matter (DOM) and flux of particulate organic matter (POM) to the seafloor. (C) The rise of eukaryote plankton increases planktonic primary production and marks a shift towards organic matter cycling dominated by POM and the eventual shrinking of a large DOM pool.

Outstanding questions

Given that the nonphotosynthetic closest relatives of cyanobacteria have been identified almost entirely from metagenomic inferences, what are the metabolic and physiological capabilities of these sister groups?

How taxonomically diverse are the earliest lineages of oxygenic phototrophs?

How has genome evolution changed across taxa?

How did the establishment of planktonic cyanobacteria (both nonsymbiont and symbionts) impact modes of carbon and other biogeochemical cycling?

Can we derive analogues for the role of cyanobacteria in past biogeochemical cycles from the modern ocean?

Concluding remarks

Ancestral cyanobacteria can be traced back to the Archean Eon, well before the GOE, but molecular phylogenetic studies show that most modern cyanobacterial clades, including the lineage leading to chloroplasts, diversified after the GOE. Cyanobacteria were the dominant primary producers during most of the Proterozoic Eon, an interval nearly two billion years in duration. Renewed biospheric transformation in the late Neoproterozoic Era is associated with the evolution of modern planktonic cyanobacteria as well as the rise of phytoplanktonic algae to global prominence. In combination, the molecular and geologic records suggest a planetary history of coevolution, with cyanobacteria and the physical Earth influencing each other repeatedly through time.

Acknowledgements

Funding support for this work came from a Royal Society University Research Fellowship to P.S.-B. and a University of Bristol Scholarship for G.B. J.D.W. acknowledges support from an AXA Research Fund Postdoctoral Fellowship.

Declaration of interests

There are no interests to declare.

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